

CLAIMS:

1. A method of linking nucleic acid components in a predetermined order to produce a nucleic acid multicomponent construct, comprising:

5 (a) providing nucleic acid components, each comprising at least one genetic element providing a functionality and at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to a terminal sequence in a separate nucleic acid component so as to allow for specific annealing and linkage of the nucleic acid components in a predetermined order;

10 (b) incubating the nucleic acid components under conditions which allow for specific annealing and linkage of the components to thereby produce the nucleic acid multicomponent construct.

15 2. The method of claim 1, wherein one or more of the nucleic acid components provide a single functionality.

3. The method of claim 1, wherein one or more of the nucleic acid components provide multiple functionalities.

20 4. The method of claim 1, wherein each of the nucleic acid components are flanked by at least one single stranded terminal sequences.

5. The method of claim 1, wherein at least one of the single stranded terminal sequences are non-palindromic.

25 6. The method of claim 1, wherein the nucleic acid components are incubated simultaneously.

7. The method of claim 1, wherein the nucleic acid components are incubated in a step-wise fashion.

8. The method of claim 1, wherein the nucleic acid components are linked directly via annealing of 5' complementary terminal sequences.

9. The method of claim 1, wherein the nucleic acid components are linked directly via annealing of 3' complementary terminal sequences.

10. The method of claim 1, wherein the nucleic acid components are linked indirectly via a linking nucleic acid molecule, the linking nucleic acid molecule comprising an oligonucleotide.

11. The method of claim 1, wherein the nucleic acid components are linked indirectly via a linking nucleic acid molecule, the linking nucleic acid molecule comprising an adaptor molecule, the adaptor molecule having terminal sequences that are complementary with 5' or 3' terminal sequences in separate nucleic acid components.

12. The method of claim 1, wherein the unique single stranded, non-palindromic terminal sequences have a length of 10 bases.

13. The method of claim 1, wherein the unique single stranded, non-palindromic terminal sequences have a length of 20 bases.

14. The method of claim 1, wherein steps (a) and (b) are repeated with one or more of the nucleic acid components substituted with a different nucleic acid component chosen from a category of components, having the same functionality or characteristic utility, but possessing the same terminal sequences which allow for linkage and production of a different nucleic acid construct.

15. The method of claim 1, wherein the nucleic acid component encodes a biological functionality selected from the group consisting of origin of replication, selectable marker, transcriptional regulatory element, structural gene or fragment thereof, transcription termination signal, translational regulatory sequence, regulators of mRNA stability, cellular localization signal, recombination elements, mutagenized genes, protein domain encoded regions, synthetic multiple cloning sites, unique restriction enzyme or DNA cleavage sites, and site for covalent or non covalent attachment of a biological or chemical molecule.
- 10 16. The method of claim 15, wherein the DNA cleavage site is part of a multiple cloning site.
- 15 17. The method of claim 1, wherein the nucleic acid component is covalently or non-covalently modified.
18. The method of claim 17, wherein the modification is biotinylation.
19. The method of claim 17, wherein the modification is fluorescent tagging.
- 20 20. The method of claim 17, wherein the modification is incorporation of polypeptide nucleic acids (PNA).
21. The method of claim 17, wherein the modification is covalent or non-covalent conjugation of a protein involved in nucleic acid modification.
- 25 22. The method of claim 21, wherein the protein involved in nucleic acid modification is an enzyme.

23. The method of claim 17, wherein the modification is covalent or non-covalent conjugation of a protein or another molecule or ion which enables the recognition and binding of a specific molecular target.

5 24. The method of claim 23, wherein the specific molecular target is a hapten.

25. The method of claim 1, wherein annealing and linkage of step (b) is achieved by heating, followed by cooling down to an appropriate temperature, such that efficient annealing of the nucleic acid component terminal sequences occurs.

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26. The method of claim 25, further comprising treating with T4 DNA ligase to ligate the nucleic acid components.

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27. The method of claim 1, wherein the nucleic acid construct is selected from the group consisting of a vector, a cDNA library, a phage or viral genome, and a gene or gene fragment.

28. The method of claim 27, wherein the gene is a mutagenized gene.

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29. The method of claim 27, wherein the gene is a combined fusion gene.

30. The method of claim 27, wherein the gene is an artificial gene.

31. A method of producing a vector, comprising:

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a) providing nucleic acid components and optionally a linking nucleic acid molecule to be assembled into the construct, each component comprising a double stranded nucleic acid molecule having at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to either a terminal sequence in a separate nucleic acid component or to a sequence in a linking nucleic acid molecule so as to allow

for specific annealing and linkage of the components in a predetermined order, wherein the nucleic acid components encode:

- i) an origin of replication
- ii) a selectable marker
- 5 iii) an insert of interest;

(b) incubating the nucleic acid components under conditions which allow for specific annealing and linkage of the nucleic acid components to thereby produce the functional vector.

10 32. The method of claim 31, for producing a cosmid vector, further comprising providing a nucleic acid component encoding a lambda phage cohesive end (cos site).

15 33. The method of claim 31, for producing a lambda phage vector, further comprising providing nucleic acid components encoding a left and a right arm of the lambda phage genome.

20 34. The method of claim 31, for producing a retroviral vector, further comprising providing a nucleic acid component encoding a retroviral genome including long terminal repeats (LTR).

25 35. The method of claim 31, for producing a yeast artificial chromosome, further comprising providing nucleic acid components encoding a yeast centromere and two yeast telomeres.

30 36. The method of claim 31, for producing a vector expressing a protein of interest, further comprising providing a nucleic acid component encoding a structural gene of interest.

37. The method of claim 31, for producing a vector expressing a cDNA library further comprising, providing nucleic acid components encoding a collection of cDNA molecules derived from poly(A)+ mRNA.

5 38. The method of claim 31, for producing a vector expressing a genomic library, further comprising providing nucleic acid components encoding a collection of gene or gene fragments derived from the genome of an organism.

10 39. A kit for the production of nucleic acid multicomponent constructs, comprising a package containing nucleic acid components, each component comprising a double stranded nucleic acid molecule having at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to either a terminal sequence in a separate nucleic acid component or to a sequence in a linking nucleic acid molecule so as to allow for specific annealing and linkage of the components in a predetermined order.

15 40. A kit for the production of nucleic acid multicomponent constructs, comprising at least 3 different nucleic acid components appropriately phosphorylated for ligation, the kit further comprising a ligase enzyme.

20 41. A kit for the production of vectors, comprising nucleic acid components, each component comprising a double stranded nucleic acid molecule having at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to either a terminal sequence in a separate nucleic acid component or to a sequence in a linking nucleic acid molecule so as to allow for specific annealing and linkage of the components in a predetermined order, wherein the nucleic acid components encode:
25 i) an origin of replication, and ii) a selectable marker

42. A method of linking nucleic acid components in a predetermined order to produce a nucleic acid multicomponent construct, comprising:

30 (a) providing the nucleic acid components and one or more linking nucleic acid molecules to be assembled into the construct, each nucleic acid component comprising a

double stranded nucleic acid molecule having at least one single stranded 5' or 3' terminal sequence, the terminal sequence having sufficient complementarity to a sequence in a linking nucleic acid molecule so as to allow for specific annealing of complementary sequences and linkage of the components in a predetermined order;

- 5 (b) incubating the nucleic acid components under conditions which allow for the specific annealing and linkage of the nucleic acid components to thereby produce the nucleic acid multicomponent construct.

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